Outcome of B-Cell Acute Lymphoblastic Leukemia in Brazilian Children: Immunophenotypical, Hematological, and Clinical Evaluation

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Summary: The aim of this study is to investigate the clinical, hematological, and immunophenotypic characteristics of Brazilian children with B-cell acute lymphoblastic leukemia (B-ALL) to identify prognostic biomarkers of the disease. Thirty-three children newly diagnosed with B-ALL were followed between March 2004 and December 2009. Information about the demographic profile, diagnosis, immunophenotype, clinical manifestations, and disease outcome were gathered from the patients' medical records. Of the 33 patients with B-ALL, 18 were male and 15 female. Eighteen patients were classified as high risk; 13 as low risk, and 2 as true low risk. The frequencies of cluster of differentiation (CD)10, CD19, and CD20 antigens were 69.7%, 81.8%, and 18.2%, respectively. Six patients (18.2%) had aberrant expression of myeloid antigens. At diagnosis, patients immunopositive for CD20 had elevated white blood cell counts ($P = 0.018$) and lower platelet counts ($P = 0.017$). The 6-year overall survival was $67.5\% \pm 3.4\%$. Our results demonstrate the distinct immunophenotypic and prognostic characteristics of patients with B-ALL, which can be related to the Brazilian racial admixture. Consequently, these results will most likely aid in the selection of additional prognostic markers and their use in monitoring the clinical manifestations and treatment response among B-ALL patients.

Key Words: acute lymphoblastic leukemia, immunophenotype, childhood

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The acute lymphoblastic leukemia (ALL) is the most common childhood malignancy, with a high incidence in the age range of 2 to 5 years.¹ The diagnosis is established based upon the patients' clinical signs and symptoms and the morphologic and immunophenotypic analyses of bone marrow (BM) and peripheral blood (PB) cells to

identify the cell lineage associated with the disease. The clinical presentation of ALL at diagnosis is nonspecific, and most patients may experience fatigue, fever, infections, bleeding, and lymph node enlargement secondary to the proliferation of blast cells in the BM, PB, and organs such as the spleen, liver, central nervous system (CNS), and testicles.² The treatment of ALL has improved over the years, with survival and cure rates reaching approximately 90% as a result of specific treatment approaches and protocols.^{3,4} Those protocols are based on a classification of patients according to risk categories, taking into account data related to white blood cell (WBC) count at diagnosis, age, CNS involvement, and the type of leukemic cell.

The importance of the analysis of the immunophenotypic markers for the classification of leukemia into subgroups of clonal cells is well established. Several studies have highlighted their relevance in the ALL prognosis, whereas others are still a matter of debate.^{5–8} The aim of this study was to characterize the clinical, hematological, and immunophenotypic features of childhood B-cell ALL (B-ALL) leukemia, evaluate the prognostic markers, and the outcome of this group of patients treated with a medication regimen specific to ALL.

MATERIALS AND METHODS

Patients

A cohort of 33 pediatric patients newly diagnosed with B-ALL was followed between March 2004 and December 2009 at ONCO, Bahia Oncology Society Ltd. These patients were treated according to the protocol of The Brazilian Childhood Leukemia Treatment Group (GBTLLI-LLA 93)⁹ from the Brazilian Society of Pediatric Oncology. Patients with T-cell ALL and those who had been treated with another treatment schedule were excluded from the study. This study was approved by the human subject research board of the Centro de Pesquisa Gonçalo Moniz-Fundação Oswaldo Cruz (CPqGM-FIOCRUZ). The parents or guardians of the study patients provided written signed informed consent, in accordance the Brazilian standards for the development of research on humans, and in accord to the Helsinki Declaration of 1975, as well as its revision.

Medical Records

Clinical information, demographic profile, diagnosis, immunophenotype of the immunoblasts, treatment outcome, and data about the disease from all 33 children with B-ALL were collected from their medical records.

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Risk Classification

According to the GBTLI-LLA 93 protocol, the patients were classified as follows: true low risk (TLR), which was defined by an age range equal or greater than 1 and equal or under than 10 years, initial WBC count $\leq 10 \times 10^9/L$, absence of mediastinal tumors, absence of CNS leukemia, hepatomegaly, and splenomegaly < 5 cm; low risk (LR), which was defined by an age range of equal or greater than 1 and equal or under 10 years, initial WBC count between 10 and $50 \times 10^9/L$, and/or presence of mediastinal tumor and/or hepatomegaly and splenomegaly ≥ 5 cm; and high risk (HR), which was defined as age below 12 months and above 10 years, and/or initial WBC count $> 50 \times 10^9/L$ and/or CNS leukemia.

GBTLI-LLA 93 Treatment Protocol

All patients were treated using the GBTLI ALL-93 protocol according to their risk stratification (Table 1).

CNS prophylaxis by triple intrathecal therapy with methotrexate, cytosine arabinoside, and dexamethasone was administered to all patient groups. The dosage of each drug, by age, is described in Table 2. Cranial irradiation (18 Gy) was administered only to the HR group during the reinduction phase.

Evaluation of Response

BM specimens were analyzed during the induction therapy at day 7 (D7), day 15 (D15), and day 28 (D28), and PB samples were examined on day 1 (D1), day 7 (D7), and day 14 (D14). Patients were considered fast responders if their WBC counts were $< 5 \times 10^9/L$ at D7, with absence of blasts on PB smears at D7 and D14, and $< 25\%$ of blasts in the BM at D15. Slow responders were defined by WBC counts $\geq 5 \times 10^9/L$ at D7, with PB blasts at D7 and D15, and BM specimens with $> 25\%$ of leukemic blasts. Non-responders were patients which demonstrated $> 5\%$ of

TABLE 1. GBTLI ALL-93 Treatment Protocol

Induction phase—high risk, low risk, and true low risk (week 0-4)	
DEXA	6 mg/m ² /d $\times$ 28 (PO)
VCR	1.5 mg/m ² weekly $\times$ 4 (IV)
DAUNO	25 mg/m ² weekly $\times$ 4 (IV)
L-ASP	10,000 IU/m ² $\times$ 4 (IM)
ARA-C	HR: 750 mg/m ² $\times$ 6 + L-ASP 6000 IU/m ²
	LR/TLR: 75 mg/m ² $\times$ 8 (SC)
	IT $\times$ 2 (LR, TLR); IT $\times$ 4 (HR)
MTX/ARA-C/DEXA	
Induction phase 2 (week 4-6)	
L-ASP	10,000 IU/m ² every 2 d $\times$ 8 (LR, TLR)
	6000 IU/m ² $\times$ 6 (HR) (IV)
ARA-C	75 mg/m ² D29-D32, D40-D43 (LR, TLR)
	750 mg/m ² (12/12 h) $\times$ 3 (D36-D38) (HR) (SC)
	IT $\times$ 1 (D43)
MTX/ARA-C/DEXA	
Circumstantial plan	
Teniposide	165 mg/m ² twice weekly $\times$ 6 (IV)
ARA-C	300 mg/m ² twice weekly $\times$ 5 (IV)
Intensification phase—high risk, low risk, and true low risk (week 7-14)	
MTX	2000 mg/m ² $\times$ 4 (IV)
6-MP	50 mg/m ² /d (PO)
MTX/ARA-C/DEXA	IT $\times$ 4
Reinduction phase—high risk, low risk, and true low risk (week 15-21)	
DEXA	6 mg/m ² $\times$ 21 (PO)
VCR	1.5 mg/m ² $\times$ 4 (IV)
L-ASP	10,000 IU/m ² $\times$ 4 (IM)
6-MP	50 mg/m ² $\times$ 14 (PO)
ARA-C	75 mg/m ² $\times$ 8 (SC)
MTX/ARA-C/DEXA	IT $\times$ 3 (LR, TLR); IT $\times$ 5 (HR)
Maintenance phase—high risk (week 22-77)	
Block A (6 cycles)	
ARA-C	750 mg/m ² (12/12 h) $\times$ 8 (IV)
L-ASP	6000 IU/m ² /cycle (IM)
Block B (6 cycles)	
Prednisone	40 mg/m ² $\times$ 15 (PO)
VCR	1 mg/m ² /wk $\times$ 3 (IV)
Block C (6 cycles)	
6-MP	75 mg/m ² $\times$ 21 (PO)
MTX	40 mg/m ² /wk $\times$ 3 (IM)
Maintenance phase—high risk (week 78-130), low risk, and true low risk (week 22-130)	
6-MP	50 mg/m ² /d (PO)
MTX	25 mg/m ² /wk (IM)
MTX/ARA-C/DEXA	IT every 8 wk (except for HR)
VCR	1.5 mg/m ² every 8 wk (IV) (except for HR)
DEXA	4 mg/m ² $\times$ 7 every 8 wk (PO) (except for HR)

6-MP indicates 6-mercaptopurine; ARA-C, cytosine arabinoside; DAUNO, daunorubicin; DEXA, dexamethasone; HR, high risk; IM, intramuscular; IT, intrathecal; IV, intravenous; L-ASP, L-asparaginase; LR, low risk; MTX, methotrexate; PO, per oral; TLR, true low risk; VCR, vincristine.

TABLE 2. Intrathecal Drug Dosage by Age

	Age (y)			
	< 1	1 ≤ Age < 2	2 ≤ Age < 3	≥ 3
Methotrexate (mg)	6	8	10	12
ARA-C (mg)	20	30	50	70
DEXA		2 mg/m ² (maximum dosage: 2 mg)		

ARA-C indicates cytosine arabinoside; DEXA, dexamethasone.

blasts in the BM or extramedullary leukemia at the end of the induction therapy. Complete remission (CR) was defined as <5% lymphoblast in a normocellular BM in the absence of clinical evidence of disease, with neutrophil count >0.5 × 10⁹/L and platelet count ≥ 75 × 10⁹/L in the PB. Disease relapse was characterized by the recurrence of blasts ≥ 5% in the BM or evidence of CNS leukemia or testicular infiltration by blast cells.

Statistical Analyses

Patient data were analyzed using software SPSS 17 (SPSS Inc., Chicago, IL) and GraphPad Prism 5 (GraphPad Software, San Diego, CA). Statistical differences were considered significant at *P* values <0.05.

Qualitative data were analyzed by the χ^2 test and Fisher exact test and included all cluster of differentiation (CD) antigens for cytoplasmic and membranous markers using a panel composed of CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD9, CD10, CD13, CD14, CD19, CD20, CD22, CD33, CD34, CD38, CD45, CD56, CD58, CD79a, CD135, cCD3, cCD13, cCD22, cCD79a, mCD22, cIgM, TdT (terminal deoxynucleotidyl transferase), HLA-DR and MPO (myeloperoxidase). Clinical symptoms, including hepatomegaly, splenomegaly, fever, anemia, articular pain, petechial rash, renal lithiasis, and evidence of CNS leukemia or testicular infiltration by blast cells were analyzed to determine the categories of risk. For the purpose of statistical analysis, patients in the LR and TLR groups were combined into a single group of LR patients. Quantitative data were expressed as the mean ± SD and median, and the distribution of quantitative variables was determined using the Kolmogorov-Smirnov test. Mean values of quantitative variables were compared using the *T* test for unpaired data with normal distribution and Mann-Whitney test for non-normally distributed data.

The overall survival (OS) of patients was calculated from the beginning of therapy to the date of the last follow-up by the Kaplan-Meier method.

RESULTS

Patients' Characteristics

Of the 33 patients with B-ALL, 18 (54.6%) were male and 15 (45.4%) female, with a ratio male/female of 1.2:1, and an age range at diagnosis of 4.4 ± 2.98 years. The median of WBC counts at diagnosis was 7 × 10⁹/L (range, 1.4 to 513 × 10⁹/L) and the median platelet counts was 35 × 10⁹/L (range, 6 to 327 × 10⁹/L); only 1 patient had WBC count >100 × 10⁹/L. Patient characteristics at diagnosis are summarized in Table 3. Eighteen (54.5%) patients were classified as HR, 13 (39.4%) patients as LR, and 2 (6.1%) patients as TLR.

Signs and symptoms most commonly observed in the ALL studied patients were hepatomegaly (66.7%), splenomegaly (57.8%), lymphadenopathy (57.8%), fever (29.7%),

anemia (16.2%), joint pain (8.1%), and petechiae (5.4%). The mean time between onset of symptoms and diagnosis was 38 days (range, 7 to 180 d).

The expression of cell surface antigens was considered positive when present on >20% of the blast cells. The frequencies of the CD10, CD19, CD20, and CD34 antigens were 69.7% (23/33), 81.8% (27/33), 18.2% (6/33), and 30.3% (10/33), respectively. Six patients (18.2%) had aberrant expression of myeloid antigens (CD13 and/or CD33).

Treatment Outcome

Twenty-one patients were considered fast responders (63.6%) and 12 patients slow responders (36.4%). After the induction phase (D28), 8 patients (24.2%) were considered nonresponders. Seven of them were classified as HR and 1 as LR. They were treated with induction phase 2 and at D43 all of them still presented >5% of blasts in BM. They were treated subsequently with circumstantial plans and then achieved CR. Five (15.1%) patients, who achieved CR, experienced relapse of their disease, including 3 on-therapy and 2 off-therapy, with a median relapse time of 28.5 months (range, 8 to 46 mo). Three of them had combined relapse in the BM and CNS, but none of these patients had CNS infiltration at diagnosis. At the end of the follow-up period, 28 patients were living and off-treatment, and 5 (15%) patients died from relapse of their disease. There were no deaths during the induction phase. None of

TABLE 3. Characteristics of Patients at Diagnosis

Characteristic	N (%)
Sex	
Male	18 (54.6)
Female	15 (45.4)
Age (y)	
< 1	1 (3.0)
1-10	29 (87.9)
> 10	3 (9.1)
WBC (× 10 ⁹ /L)*	
< 10	15 (45.4)
10-50	14 (42.4)
> 50	2 (6.1)
Platelet count (× 10 ⁹ /L)	
< 20	8 (24.2)
20-100	14 (42.4)
> 100	9 (27.3)
Hemoglobin (g/dL)	
< 7	10 (30.3)
7-11	19 (57.6)
> 11	4 (12.1)
CNS infiltration	5 (15.1)
Common ALL subtype	20 (60.6)

*Two patients lacked initial WBC and platelet counts.

ALL indicates acute lymphoblastic leukemia; WBC, white blood cell.

FIGURE 1. White blood cell (WBC) and platelet counts in patients with CD20⁺, CD20⁻, CD10/19⁺, and CD10/19⁻ B-cell acute lymphoblastic leukemia at diagnosis.

the clinical and laboratory data correlated significantly with relapse or death (data not shown).

At diagnosis, patients with CD20 positivity had higher WBC counts, with median of $19.1 \times 10^9/L$ compared with $6.5 \times 10^9/L$ ($P = 0.018$), but lower platelet counts, with $13 \times 10^9/L$ compared with $47 \times 10^9/L$ ($P = 0.017$) for CD20-immunonegative patients. Patients who expressed both CD10 and CD19 had higher WBC counts and lower platelet counts with $P = 0.033$ and 0.028 , respectively (Fig. 1). Patients with CD10 immunopositivity had lower platelet counts at D1, D7, and D15, which was statistically significant ($P = 0.010$) at D15.

The median platelet counts at D1, D7, and D15 were significantly lower ($P = 0.020$, 0.010 , and 0.008 , respectively) in patients who presented with hepatomegaly at diagnosis (66.7%) than in those who did not.

Cytogenetic and molecular genetic analyses were not required in the GBTLI LLA-93 protocol, but 2 patients were evaluated for the presence of common translocations and 1 of them was found to have $t(12; 21)(p13; q22)$, which results in the TEL-AML1 rearrangement (ETV6-RUNX1). He was classified as HR and fast responder, achieved CR and was living at the end of the study.

One patient had a WBC count of $513.5 \times 10^9/L$ and CNS leukemia at diagnosis, being classified as HR. The time between onset of symptoms and diagnosis was 180 days. Despite being classified as fast responder, the patient relapsed 8 months after beginning therapy, and succumbed 2 days later.

The 6-year OS of the cohort of patients was $67.5\% \pm 3.47\%$ and demonstrated no statistically significant association with clinical, hematological, and immunophenotypic data.

DISCUSSION

The ALL is the most common malignancy of childhood with a peak incidence between 2 and 5 years of age. Despite the lack of official data about the incidence of ALL in the Brazilian state of Bahia, an incidence rate of 59 cases per year for all childhood leukemia has been reported.¹⁰ Robazzi et al¹¹ described an incidence rate of 26 to 31 cases per year for ALL followed at ONCO, with B-ALL representing about 85% of all cases. Thus, we studied a convenience sampling of 33 patients followed at a single oncology center that was the main reference center for diagnosis and treatment of childhood cancer in the state of Bahia, representing approximately 10% of all cases of B-ALL diagnosed in Bahia between 2004 and 2009. In the present study, the ratio of male to female was 1.2:1, which is in agreement with incidences of ALL worldwide that have been frequently described as higher among males than female. The signs and symptoms described for the patients included here were broadly similar to other populations.^{12,13}

The common ALL antigen, CD10, was expressed in 69.7% compared with 94% of blast cells according to Pui et al.⁸ Similar differences were found in the frequency of CD20 antigen, which was expressed in 18.2% of blast cells from the studied patients with ALL compared with 48% according to Jeha et al.⁵ Those differences may be attributed to the racial mix of the Brazilian population, representing one of the most heterogeneous in the world. This diversity is composed by the Portuguese colonizers, autochthonous Amerindians, Africans (brought to Brazil as slaves) in addition of immigrants of several parts of the world, like Germany, Italy, Spain, and Japan.¹⁴ Alves-Silva et al¹⁴ developed a study based on mitochondrial DNA to

characterize the Brazilian population genetic background, and described a proportion of 33% of Native American, 28% of African, and 39% of European in Brazilian mitochondrial DNA pool; these results are in accordance with a more recent study that demonstrate the strong European genetic background in this population group.¹⁵ Differences in incidence and treatment outcome in ALL was described, showing the African American descent with the lower incidence of ALL but with the inferior treatment outcome as compared with European American, representing both genetic and nongenetic factors that influences ALL incidence and outcome.¹⁶ Despite of some, there is still a lack of studies evaluating racial and ethnics aspects in ALL biology. The expression of CD10 was not associated with survival or prognosis among the studied patients with ALL as reported by Pui et al.⁸

The expression of CD20 antigen has been associated with a poor prognosis in adult ALL patients, unlike of pediatric patients. Jeha et al⁵ suggested an association of the CD20 expression with a lower WBC count at diagnosis and a good clinical outcome of ALL in childhood. In the present study CD20⁺ patients were associated with a higher WBC and lower platelet counts at diagnosis, suggesting an increased risk for relapse. Mannelli et al¹⁷ evaluated the prognostic significance of CD20 in 172 B-ALL patients treated within the multicenter NILG 09-2000 study (MRD-oriented). Only 52 (30.2%) patients were CD20⁺ and they failed to demonstrated prognostic significance of this surface marker in context of the MRD-based prognostic analysis. Solano-Genesta et al¹⁸ evaluating the CD20 in 93 pediatric leukemia patients found an expression of 62% and these patients presented a slightly, but not significant marker expression, less OS in CD20⁺ group, and similarly more relapses, but also nonsignificant. Another study by Naithani et al¹⁹ evaluated the prognostic significance of CD20⁺ in 259 patients MRD-based treated, among that 51% expressed CD20 and there is no association with prognosis. In the present study we failed to demonstrate the relationship of CD20 with prognosis or survivor.

In our study, the same association observed with CD20, was observed with patients who expressed both CD10 and CD19. Glencross et al,²⁰ studying children with ALL CD19⁺/CD10⁺, hypothesized that the low density of CD10 among black individuals and in males is most likely associated with poor prognosis. This study did not describe any statistically significant correlation among WBC count, immunophenotype, risk category, initial CNS leukemia, and relapse or survival, most likely due to the small number of patients in the cohort.

In the present study, 5/33 (15.1%) patients died, with no death occurring during the induction phase, in contrast with data reported by Marwaha et al²¹ and Arya et al²² in India. In these 2 studies, 128/532 (24.1%) and 61/254 (24.0%) patients with ALL died, with 68 (12.8%) and 28 (11%) deaths during induction and 60 (11.3%) and 33 (14.8%) deaths during remission, respectively. In our cohort all patients received supportive care as hydration, proper management of tumor lysis syndrome, availability of blood transfusions, and treatment with antibiotics when necessary. Furthermore, patients classified as HR initiated induction therapy in the intensive care unit. Eight patients (24.2%) were classified as nonresponders at the end of induction, so they continue to phase 2 of induction and circumstantial plan and finally achieved CR. Since the GBTLI-93 protocol does not require cytogenetic and molecular genetic neither MRD

analyses, we do not know if that information would explain this observed nonresponse rate, whereas there is no non-compliance within the induction therapy.

Our data concerning patients' OS were very similar with data from these developing countries, and lower than data from several US and Europe studies, where the patients attained >80% survival. This is most likely due to the difficulty experienced by patients in accessing the health system for early diagnosis and treatment all over the state of Bahia. It may influence the identification of more severe disease in this population.

Knowledge of the biology of this disease and the development of risk-directed treatment protocols are critical for the success in the battle against ALL, by minimizing relapses and chemotherapy-associated deaths. In addition, some factors must be considered in the analysis of these data, such as the socioeconomic and demographic characteristics of the studied population located in Northeast Brazil, an area with extensive social inequity presenting a lack of specialized professional and reference centers for the diagnosis of ALL. For these reasons, the majority of patients delay their treatment because they have to travel to large cities, often far from their hometown, being hosted by public institutions, which, unfortunately, are not able to meet all the demand; consequently, patients often neglect treatments, thereby reducing their chances for a cure. Our results show distinct immunophenotypic and prognostic characteristics from the studied patients with ALL, which can be related to the Brazilian racial admixture. It may help to select possible prognostic markers and use them for the clinical monitoring and treatment response of these patients. However, the data related to OS are consistent with those obtained in developing countries, such as India and China. These data need to be confirmed by a large multicenter study involving more Brazilian states with different socioeconomic characteristics to describe the diversity of the disease, standardize protocols, and contribute to improving government policies related to the diagnosis and treatment of pediatric ALL in Brazil.

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